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Original Article

Modulation of Tuberculosis Pathway by *Phaseolus vulgaris*: An *in silico* Approach

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ABSTRACT

Aim/Background: Traditionally, *Phaseolus vulgaris* is used to treat common diseases. However, the affinity of its phytocompounds with proteins involved in the pathogenesis of Tuberculosis (TB) has not yet been illuminated. The present study was designed to elucidate the molecular mechanisms of *Phaseolus vulgaris* and its antituberculosis activity via compound-gene set pathway enrichment analysis, network pharmacology, docking studies and molecular dynamics simulation. **Materials and Methods:** The phytocompounds were retrieved from herbal databases along with the structural information for each compound to investigate the druggable characteristics that were predicted using MolSoft. The phytocompound toxicity was assessed using Protox, while compounds targeting TB-related proteins were identified by Gene Card and Omim Database targets. STRING and KEGG pathways analyzed the molecular modulations. Interactions between compounds, proteins and pathways were visualized using Cytoscape 3.6.1, while the docking of compounds with protein targets was performed using AutoDock 4.2 and molecular dynamic simulation using GROMACS 23.2 for 200 ns. **Results and Conclusion:** A total of 11 phytocompounds of *Phaseolus vulgaris*, obeying the rule of five, modulated 11 TB pathways. Naringenin and naringenin-7-glucoside had the highest drug-likeness scores, while Teasterone[a] and Clerosterol showed a top binding affinity with MAPK3. This study offers molecular insights for future wet-lab exploration of *Phaseolus vulgaris* compounds against TB.

Keywords: Tuberculosis-Related Pathways, Gene Set Enrichment, Molecular Dynamics Simulation, Network Pharmacology, *Phaseolus vulgaris*.

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INTRODUCTION

Tuberculosis (TB), caused by the bacterium Mycobacterium tuberculosis, continues to pose a significant global health threat despite decades of concerted efforts aimed at its control and eradication.[1] As one of the oldest known infectious diseases affecting humans, TB remains a major cause of morbidity and mortality worldwide, particularly in low- and middle-income countries. The World Health Organization (WHO) estimates that approximately 10 million people developed TB in 2020, with nearly 1.5 million succumbing to the disease.[2] The clinical manifestations of TB are diverse, with pulmonary TB being the most common form. The disease primarily affects the lungs but can also spread to other organs, causing extrapulmonary TB.[3] The typical symptoms of active pulmonary TB include persistent cough, fever, night sweats, weight loss and haemoptysis.[4] TB is transmitted through the inhalation of respiratory droplets containing the infectious bacilli and its progression depends on a complex interplay of factors, including the individual's immune status, the virulence of the bacterium and environmental influences.[5]

The emergence of drug-resistant strains of Mycobacterium tuberculosis, especially Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) strains further complicates TB management. These strains are less responsive to standard TB treatment regimens, increasing the risk of treatment failure and the spread of resistant strains within communities. The global response to TB includes strategies for early diagnosis, proper treatment and infection control.[6] While the advent of Directly Observed Treatment Short-course (DOTS) programs has improved treatment outcomes, challenges remain in ensuring

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universal access to accurate diagnostic tools, appropriate medications and comprehensive healthcare infrastructure.[7] In Ayurveda, traditional herbs are utilized to treat diseases like tuberculosis by local healers and physicians. Herbal supplementation increases overall survival in TB patients and reduces the negative effects of conventional therapy, according to a new meta-analysis of randomized controlled trials. The common bean, *Phaseolus vulgaris*,[8] is a versatile and extensively grown species of legume. It comes from Central and South America and has long been a mainstay of many different civilizations. The plant produces edible seed pods that can be cooked or consumed in a variety of ways.[9] Both conventional and contemporary agriculture depend on it as a crop due to its nutritional value, flexibility and capacity to fix nitrogen in the soil.[10] Chickpeas, a plant with bioactive components, offer health benefits like analgesic, anti-inflammatory, antibacterial, antidiabetic, diuretic, antioxidant and anti-obesity properties.[11] To further understand the molecular mechanism of *Phaseolus vulgaris*, researchers applied compound gene set pathway enrichment and network pharmacology techniques. Network pharmacology studies the influence of medications on the illness as a whole, taking a system-wide approach. Systems biology, network analysis, connectivity, redundancy and pleiotropy are all addressed in this method. The network pharmacological platform has been successfully used to screen beneficial components and find herb pharmacological processes.[12]

The current study aims to elucidate the molecular mechanism of action of potential bioactive phytoconstituents of *Phaseolus vulgaris* for their anti TB activity through target identification, compound-gene set enrichment analysis, network pharmacology and *in silico* docking studies. The main strength of network pharmacology is its ability to choose and understand the synergistic effects of bioactive substances by looking at how they affect many disease pathways. In structural molecular biology and computer-assisted drug creation, molecular docking is a crucial tool. Docking methods that work well use a scoring system that ranks candidate dockings correctly and explores high-dimensional spaces quickly.

MATERIALS AND METHODS

Mining of Phytocompounds and Drug-Like Property Prediction

Herb databases such as IMPPAT[13] and published articles were utilized to retrieve a list of isolated compounds from *Phaseolus vulgaris*. Chemical structural information, including Molecular Weight (MW), Molecular Formula (MF), Number of Hydrogen Bond Donors (NHBD) and Acceptors (NHBA), LogP value, BBB SCORE, DLS, MollogP, MollogS, MolpsaA2, MolvolA3, canonical SMILES and chemical identification number, were retrieved from the PubChem chemical database. The canonical SMILES were then used to predict probable drug-like properties using Lipinski's rule of 5 models on the MolSoft online server (https://www.molsoft.com/mprop/).[14] Carcinogenicity, hepatotoxicity, nephrotoxicity, cardiotoxicity, etc. profiles of each compound were predicted using Protox (https://tox-new.charite.de/protox_II/).[15]

Target Identification

Canonical SMILES were queried for target prediction using Swiss target prediction (http://swisstargetprediction.ch/)[16] and STITCH (http://stitch.embl.de/),[17] corresponding to existing known therapeutic drug molecules. Furthermore, target proteins involved in tuberculosis were identified based on known tuberculosis targets reported in the Gene Cards and OMIM Database (https://www.genecards.org/ and https://www.omim.org/).[18] Finally, compound genes and disease genes were merged to identify overlapping genes using a Venn diagram(https://bioinformatics.psb.ugent.be/webtools/Venn/).[19]

Pathway and Network Analysis

The STRING database (https://string-db.org/) and KEGG pathway database (https://www.genome.jp/kegg/)[20] were utilized to understand protein-protein interactions and molecular pathways modulated by tuberculosis protein targets, respectively. The network interactions between compounds, target proteins and pathways involved in tuberculosis were constructed using Cytoscape v3.6.1 (https://cytoscape.org/) (Figure 1). The color scale and node size were used to interpret the network based on the number of edges (edge count). Nodes with the maximum number of edge counts were represented by colossal nodes.[21]

Ligand and Protein Preparation

Based on the network analysis, compounds with the highest edge count with respect to protein targets were further studied for molecular interactions. 3D structures of each compound were retrieved from PubChem[22] (https://pubchem.ncbi.nlm.nih.gov/) in structural data format (.sdf) and converted to protein data bank format (.pdb) using Discovery Studio Visualizer (https://discover.3ds.com/). Ligand preparation was performed using Autodock software, where the pdb format was converted into (.pdbqt) format for individual ligands, namely Teasterone[a], Clerosterol, Quercetin and Kaempferol. The protein structures MAPK3, AKT1, PRKCA, IGFR1, KDR were obtained from the PDB (https://alphafold.ebi.ac.uk/) (23). Individual protein structure validation was done using Ramachandran plot,[23] ensuring the selected protein quality was 94.67%,94.48%,90.54%,90.10% and 100%(https://saves.mbi.ucla.edu/) (Figure 4). To clean the binding pocket and facilitate calculations, water molecules and heteroatoms in the protein structure were removed using Discovery Studio Visualizer 2019. Protein preparation was carried out for individual proteins using Autodock software, where the protein (.pdb) format was converted into (.pdbqt) format. The chain was selected based on

its completeness of amino acid residues and the presence of the active site region.

Docking Studies

Docking studies were performed using Autodock 4.2 (https://autodock.scripps.edu/).[24] The protein was augmented with hydrogen atoms and Kollman charges and water molecules were removed to enhance the binding affinity of the macromolecule. The grid box was positioned at the active site and grid.txt and config.txt files were generated in the respective folder. The Autodock program was executed by coding the folder in the command prompt. The ligand poses with the target protein having the highest binding energy were selected for visualization of ligand-protein interactions in Discovery Studio.[25]

Molecular dynamics simulation

To conduct MDS, the software package GROMACS version 2023.2 (https://www.gromacs.org/) was utilized. Initially, the complex was prepared by removing any hetero atoms present. The protein topology was generated using the pdb2gmx module of GROMACS, applying the CHARMM all-atom force field. The ligand topology was prepared using the Swiss Param server (accessible at https:// https://swissparam.ch/) and hydrogens were added with the assistance of the Avogadro program. The intermediate complex was constructed using the GROMACS edit conf module. Subsequently, the complex was solvated in a dodecahedron box with dimensions of 1 nm on all sides, employing a three-point water model. The system was neutralized by introducing Na+ and Cl- counter ions as needed. To minimize energy, the system underwent a steepest descent integrator with a Verlet cutoff scheme for a maximum of 55000 steps, followed by application of restraints. The equilibration of the system was performed using Canonical (NVT) and Isobaric (NPT), which ensembles for 1 ns, considering two coupling groups *i.e.*, protein-ligand and water-ions. To maintain a constant volume and temperature of 300 K, a modified Berendsen thermostat (V-rescale) was utilized, while a C-rescale pressure coupling algorithm was employed to maintain a constant pressure of 1 bar. For the computation of long-range electrostatics, coulomb and Van der Waals interactions, Particle Mesh Ewald (PME) was employed with a cutoff distance of 1.2 nm. The bond lengths were constrained using the LINCS algorithm. Each complex underwent an MD simulation for 200 ns, with coordinates and energies saved every 20 picoseconds, resulting in a total of 10,000 frames. The generated trajectories were analyzed using the built-in utilities provided by GROMACS. The Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (RoG), Solvent Accessible Surface Area (SASA) total density and hydrogen bonds were extracted over the duration of 200 ns and visualized using QtGrace.[26]

RESULTS

Gene Set Enrichment and Network Pharmacology

Understanding the mechanism of action of phytocompounds from medicinal plants against diseases like tuberculosis, both

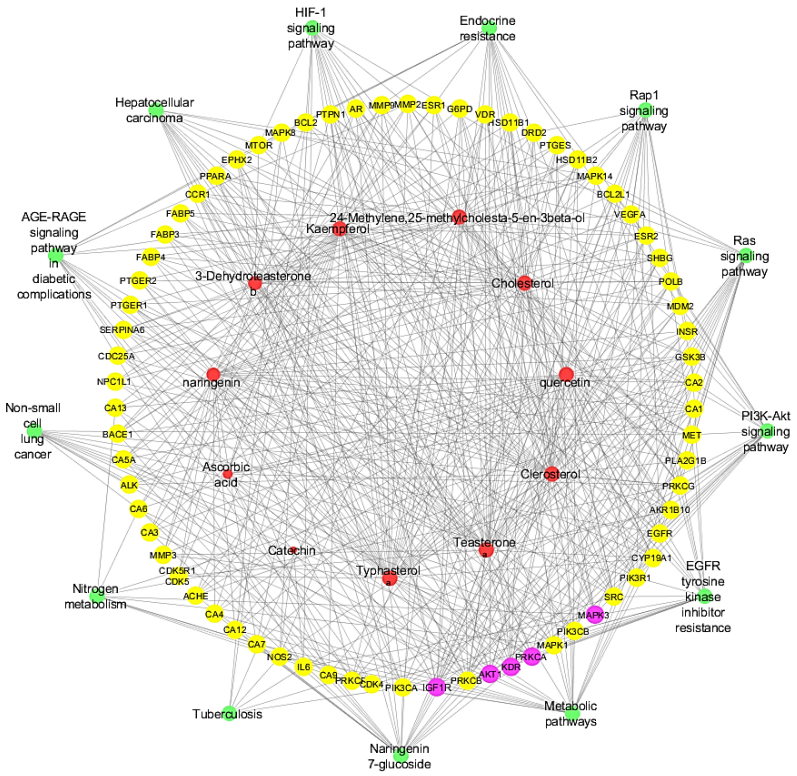

Figure 1: Network representation of compounds, proteins and pathway interactions.

in vivo and *in vitro*, can be challenging. This study utilized gene set enrichment, network pharmacology and a docking approach to elucidate the molecular mechanisms of *Phaseolus vulgaris* compounds. The phytocompounds in *Phaseolus vulgaris* were identified through herb databases and literature. A total of 151 compounds were identified in *Phaseolus vulgaris*. The selection of compounds was based on factors like oral bioavailability, half-life and drug similarity Gene Set Enrichment and Network Pharmacology.

Phaseolus vulgaris Compound-Targeting Network. A total of 151 compounds have been identified in *Phaseolus vulgaris*. Due to oral bioavailability, half-life and drug similarity properties of the compounds. Of the 151 compounds, 44 showed positive drug similarity scores.

All 11 phytoconstituents showed No violation of The Rule of Five developed by Christopher A. Lipinski,[27] which is a set of guidelines used in drug discovery and medicinal chemistry to assess the likelihood of a compound's success as an orally active drug. It helps identify compounds with favourable pharmacokinetic and physicochemical properties, which are crucial for a drug's efficacy and safety. The criteria include a molecular weight of 500 g/mol or less, hydrogen bond donor sites of 5 or less, logP (Partition coefficient) of 5 or less and hydrogen bond acceptors of 10 or less. While the Rule of Five is a guideline, it is not a strict rule and other factors also play a significant role in a compound's pharmacokinetic behaviour and drug-like properties (Tables 1 and 2) and the probable side effects of all the phytoconstituents were predicted. Ascorbic acid, Catechin and Kaempferol showed non-toxic properties like Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity and Cytotoxicity respectively (Tables 3 and 4).

The pathway enrichment analysis for 1056 targets was carried out using STRING and the KEGG pathway database. As a result, 1056 targets were identified to involve 197 distinct pathways. After peer interpretation of these pathways, 11 pathways were directly linked to the pathogenesis of Tuberculosis. Among 1056 targets, IGF1R, AKT1, PRKCA, KDR and MAPK3 were identified as therapeutic targets of TB. Among 11 compounds, teasterone[a], clerosterol, quercetin and kaempferol targeted IGf1r, AKT1, PRKCA, KDR and MAPK3. Further, these targets were involved in the HIF-1, AGE-RAGE, PI3K-Akt, Ras, Rap1 signalling pathways and EGFR tyrosine kinase inhibitor resistance, metabolic pathway, endocrine resistance, non-small cell lung cancer, hepatocellular carcinoma. These pathways were identified to play an important role in TB (Table 5).

Docking studies

Based on enrichment analysis, molecular docking of Teasterone[a] with MAPK3, IGFR1, KDR, Quercetin with AKT1, PRKCA and Clerosterol with IGFR1 was carried out. Teasterone[a] formed Non-HBI i.e. CYSA183, VALA56, ILEA48, LEUA173 and one hydrogen bond with MAPK3 i.e., META125 with the highest

Table 1: Drug-likeness and side effects of compounds.

Compound name	Compound	MF	MW	NHBA	NHBD	BBB SCORE	DLS	Mollog P	Mollog S	Molpsa A2	Molvol A3
Kaempferol	C1	$C_{15}H_{10}O_6$	286.05	6	4	2.78	0.5	1.61	-2.21	87.13	268.99
Quercetin	C2	$C_{15}H_{10}O_7$	302.04	7	5	2.55	0.52	1.19	-2.19	102.61	281.71
24-Methylene,25-methylcholesta-5-en-3beta-ol	C3	$C_{29}H_{48}O$	412.37	1	1	3.94	0.5	7.32	-6.26	16.28	537.66
Cholesterol	C4	$C_{27}H_{46}O$	386.35	1	1	4.04	0.49	7.44	-6.22	16.28	484.49
Ascorbic acid	C5	$C_6H_8O_6$	176.03	6	4	1.93	0.74	-1.59	-0.05	85.73	161.80
Clerosterol	C7	$C_{29}H_{48}O$	412.37	1	1	3.94	0.68	8.58	-6.37	16.28	524.64
Teasterone[a]	C8	$C_{28}H_{48}O_4$	448.36	4	3	2.99	0.23	4.54	-4.62	60.9	512.71
3-Dehydroteasterone b	C9	$C_{28}H_{46}O_4$	446.34	4	2	3.02	0.18	4.29	-4.57	58.53	513.24
Typhasterol[a]	C10	$C_{28}H_{48}O_4$	448.36	4	3	2.99	0.23	4.54	-4.62	60.9	512.71
Catechin	C16	$C_{15}H_{14}O_6$	290.08	6	5	2.73	0.64	0.53	-1.45	90.45	261.13
Catechin	C20	$C_{15}H_{14}O_6$	290.08	6	5	2.73	0.64	0.53	-1.45	90.45	261.13
Quercetin	C21	$C_{15}H_{10}O_7$	302.04	7	5	2.55	0.52	1.19	-2.19	102.61	281.71
Naringenin	C22	$C_{15}H_{12}O_5$	272.07	5	3	2.99	0.82	2.38	-2.81	71.98	251.12
Chlorogenic acid	C28	$C_{16}H_{18}O_9$	354.1	9	6	1.74	0.79	-0.2	-1.4	127.41	338.61
Kaempferol	C29	$C_{15}H_{10}O_6$	286.05	6	4	2.78	0.5	1.61	-2.21	87.13	268.99
Naringenin 7-glucoside	C38	$C_{21}H_{22}O_{10}$	434.12	10	6	1.7	0.83	0.06	-1.71	134.23	382.81

Table 2: ADME profile of phytocompounds.

Compound name	Compound	Mw	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Bioavailability Score
Kaempferol	C1	286.24	High	No	No	Yes	No	No	Yes	Yes	0.55
Quercetin	C2	302.24	High	No	No	Yes	No	No	Yes	Yes	0.55
24-Methylene,25-methylcholesta-5-en-3beta-ol	C3	412.69	Low	No	No	No	No	No	No	No	0.55
Cholesterol	C4	386.65	Low	No	No	No	No	Yes	No	No	0.55
Ascorbic acid	C5	176.12	High	No	No	No	No	No	No	No	0.56
Clerosterol	C6	412.69	Low	No	No	No	No	Yes	No	No	0.55
Teasterone[a]	C7	448.68	High	No	Yes	No	No	No	No	No	0.55
3-Dehydroteasterone b	C8	446.66	High	No	Yes	No	No	No	No	No	0.55
Typhasterol[a]	C9	448.68	High	No	Yes	No	No	No	No	No	0.55
Catechin	C10	290.27	High	No	Yes	No	No	No	No	No	0.55
Quercetin	C11	302.24	High	No	No	Yes	No	No	Yes	Yes	0.55
Naringenin	C12	272.25	High	No	Yes	Yes	No	No	No	Yes	0.55
Naringenin 7-glucoside	C13	434.39	Low	No	Yes	No	No	No	No	No	0.55

Table 3: Prediction.

Compound Name	Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Kaempferol	C1	Inactive	Inactive	Inactive	Inactive	Inactive
24-Methylene,25-methylcholesta-5-en-3beta-ol	C2	Inactive	Inactive	Active	Inactive	Inactive
Cholesterol	C3	Inactive	Inactive	Active	Inactive	Inactive
Clerosterol	C4	Inactive	Inactive	Active	Inactive	Inactive
Teasterone[a]	C5	Inactive	Active	Active	Inactive	Inactive
3-Dehydroteasterone b	C6	Inactive	Inactive	Active	Inactive	Inactive
Typhasterol[a]	C7	Inactive	Active	Active	Inactive	Inactive
Catechin	C8	Inactive	Inactive	Inactive	Inactive	Inactive
Quercetin	C9	Inactive	Active	Inactive	Active	Inactive
Naringenin	C10	Inactive	Inactive	Inactive	Inactive	Active
Naringenin 7-glucoside	C12	Inactive	Inactive	Active	Inactive	Inactive
Ascorbic acid	C13	Inactive	Inactive	Inactive	Inactive	Inactive

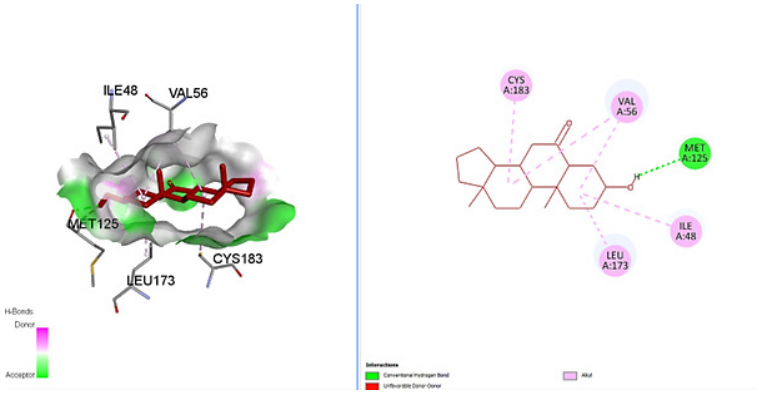

Figure 2: Interaction of Teasterone[a] with MAPK3 (a) 3D representation (b) 2D representation.

binding energy of -8.6 kcal/mol. Teasterone[a] no hydrogen bond interaction and Non-HBI i.e., TYRA168, TYRA178 with IGFR1. And showed the highest binding energy of -6.7 kcal/mol. Teasterone[a] formed Non-HBI i.e. PROA16 and one hydrogen bond KDR i.e. HISA24 with the highest binding energy of -6.3 kcal/mo. Quercetin Non-HBI i.e. LEUA29 and formed four hydrogen bond i.e LYSA179, LYSA276,THRA312, ASPA274, with the highest binding energy of -8.3kcal/mol. Quercetin formed Non-HBI i.e. ILEA610, ALAA271, PHEA261, LYSA604 and three hydrogen bond i.e. ASPA601, TYRA275, META269., with PRKCA the highest binding energy of -7.8 kcal/mol. Clerosterol no hydrogen bond Non-HBI i.e. LEUA1361, PROA1362, TYRA168, TYRA718 and showed the highest binding energy of -6.7 kcal/mol. The BE, changes in ligand orientation after docking and hydrogen bond interaction are shown in (Figures 2), (Table 6).

Molecular dynamics simulation

The Molecular Dynamic Simulation (MDS) showed stability of the Teasterone[a] with MAPK3 complex with the RMSD fluctuation in the range of ~8.2 Å to ~10.4 Å for both complex and backbone after a stability time of ~50 ns, The RMSD ranged from 0.8 to 1.39 nm throughout the MD run and after 75 ns it is noticed that complex and backbone were in the stable phase with fluctuation not more than 0.2 nm. The difference between the RMSD of the backbone and complex was 1.8 Å showing minimum fluctuation in the complex. The RMSF of the C-alpha with complex showed the highest RMS fluctuation of 23.1 Å. The RMSF value ranged from 0 to 0.5 nm throughout the run. The SASA plays important role in MD simulation which determines the decisive factor in protein folding and stability studies. SASA revealed a fluctuation in the range of ~180 nm^2 to ~205 nm^2 after the stability period and it ranged from 179 to 204 nm^2, which is under the acceptable range. The density of complex shown average 1002 kg/m^3 during the MD run. This indicates the stable interaction between the receptor and compound Teasterone[a] with MAPK3 The Radius of Gyration (RoG) displayed fluctuations in the range of ~21.7 Å to ~23.1 Å; the RoG was seen towards the higher range for ~125 ns to 145 ns, thereafter a drop of ~0.6 Å was seen which remained stable with minor fluctuations and the highest of 3 H-bond forms were observed during the MD run, however during the time period of 51-58 ns, there were no H-bond formation the (Figures 3 and 4), the supplementary data is provided in the form of Movie.

DISCUSSION

The present study traced 11 documented phytoconstituents of *Phaseolus vulgaris* to propose a probable mechanism against tuberculosis. These phytoconstituents were predicted to target proteins and involved in 197 pathways, of which 11 pathways were found to associate with tuberculosis. Based on 'Edge count,' the interaction network between phytoconstituents, protein molecules and their pathways was constructed and analyzed. IGf1r, AKT1, PRKCA, KDR and MAPK3 were identified as potential TB therapeutic targets among 1056 targets. among the 11 compounds Teasterone[a], clerosterol, quercetin and kaempferol were targeted IGf1r, AKT1, PRKCA, KDR and MAPK3. Also, these targets were shown to be engaged in the HIF-1, AGE-RAGE, PI3K-Akt, Ras and Rap1 signaling pathways, as well as EGFR tyrosine kinase inhibitor resistance, metabolic pathway, endocrine resistance, Non-small cell lung cancer and hepatocellular carcinoma. IGF1R (Insulin-like Growth Factor 1 Receptor): IGF1R is involved in cell growth, survival and proliferation. It might indirectly influence immune responses and cellular processes during TB infection, as immune cells often need to regulate their growth and activation to combat the infection effectively. AKT1 (Protein Kinase B): AKT1 is involved in a variety of biological activities, including cell survival and metabolism. In the setting of infections, it can alter immune cell survival and activation, which are critical for TB control. PRKCA

Table 4: Probability.

Compound Name	Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Kaempferol	C1	0.68	0.72	0.96	0.52	0.96
24-Methylene,25-Methylcholesta-5-En-3beta-Ol	C2	0.88	0.6	0.99	0.97	0.93
Cholesterol	C3	0.85	0.59	0.99	0.96	0.95
Clerosterol	C4	0.77	0.61	0.99	0.97	0.9
Teasterone[a]	C5	0.58	0.5	0.99	0.67	0.86
3-Dehydroteasterone B	C6	0.75	0.52	0.99	0.8	0.78
Typhasterol[a]	C7	0.58	0.5	0.99	0.67	0.86
Catechin	C8	0.72	0.51	0.96	0.55	0.84
Quercetin	C9	0.69	0.68	0.87	0.51	0.99
Naringenin	C10	0.67	0.62	0.88	0.83	0.59
Naringenin 7-Glucoside	C12	0.82	0.85	0.63	0.76	0.69
Ascorbic Acid	C13	0.86	0.92	0.99	0.87	0.65

Table 5: Pathways associated with tuberculosis progression modulated by phytocompounds.

Pathway ID	Pathway	Gene Count	FDR	Genes within pathway
hsa04014	Ras signaling pathway.	29	4.14E-13	PIK3CA IGF1R EGFR KIT PIK3CB BCL2L1 INSR PRKCB PLA2G1B MET PTPN11 ABL1 PLA2G5 MAPK8 PLA2G2A RASGRP3 FGFR1 PLA2G10 MAPK9 PRKCA PIK3R1 PGF AKT1 VEGFA VEGFA MAPK1 MAPK14 MAPK3 PRKCG KDR.
hsa01522	Endocrine resistance.	22	1.86E-15	MDM2 MAPK3 PIK3CA IGF1R EGFR PIK3CB PTK2 ESR2 MTOR JUN MMP9 SRC MAPK8 BCL2 MAPK9 ESR1 PIK3R1 AKT1 AKT1 MAPK1 FLT3 MAPK3 PRKCG KDR.
hsa04933	AGE-RAGE signaling pathway in diabetic complications.	29	3.45E-21	MMP2 SERPINE1 MAPK14 CDK4 MAPK3 NOX4 PIK3CA STAT3 PIK3CB NOS3 PRKCB PRKCE CASP3 F3 JUN PIM1 PRKCD MAPK8 BCL2 IL6 MAPK9 TNF PRKCA AGTR1 PIK3R1 AKT1 VEGFA VEGFA MAPK1 FLT3.
hsa04066	HIF-1 signaling pathway.	32	2.26E-23	MAPK1 HMOX1 SERPINE1 VHL MAPK3 PRKCG PIK3CA STAT3 IGF1R EGFR PIK3CB NOS3 INSR PRKCB NOS2 CAMK2D MTOR EGLN1 PDK1 CAMK2B LDHB BCL2 IL6 PRKCA PIK3R1 HIF1A PFKFB3 LDHA AKT1 EGLN2 VEGFA VEGFA CYP51A1.
hsa01521	EGFR tyrosine kinase inhibitor resistance.	22	6.58E-16	PIK3CA STAT3 IGF1R EGFR PIK3CB AXL BCL2L1 PRKCB MET GSK3B MTOR SRC BCL2 IL6 PRKCA PIK3R1 AKT1 VEGFA VEGFA MAPK1 MMP2 MAPK14 CDK4.
hsa04151	PI3K-Akt signaling pathway.	38	2.45E-16	CDK4 MDM2 MAPK3 KDR PIK3CA CDK6 CDK2 IGF1R EGFR KIT PIK3CB NOS3 BCL2L1 INSR YWHAG CHRM1 MET GYS1 F2R GSK3B PTK2 PKN1 PIK3CG MTOR SYK BCL2 IL6 JAK3 FGFR1 CHRM2 PRKCA RXRA PIK3R1 PGF AKT1 VEGFA VEGFA MAPK1 MAPK3 PRKCG KDR.
hsa01100	Metabolic pathways.	93	1.69E-21	PYGM CA12 HSD17B2 HMOX1 PYGL ATP12A CD38 CYP27B1 SIRT2 ALOX12 FOLH1 CYP2C9 ALDH2 TYR PIK3CA MGLL SQLE PDE3B CA2 CA3 AKR1B1 HMGCR PIK3CB CBR1 CYP11B1 NOS3 CA4 UGT2B7 CA5A GSTM1 PLA2G1B CA5B HSD11B2 GYS1 CA13 EZH2 CYP11B2 NOS2 PDE2A CYP3A4 MAOA ACACB PTGES IMPDH1 PDE4D CA7 DHCR7 ARG1 PDE3A PIK3CG DNMT1 AKR1B10 PNP PTGS1 SMYD2 HSD11B1 PTGS2 CA14 CHIA ATP6AP1 CYP17A1 SCD CYP2C19 AKR1A1 GLO1 ALOX5 PLA2G5 HSD17B3 CA6 MAOB CA9 HAO1 XDH AKR1C4 AKR1C3 G6PD TYMP LDHB CYP19A1 AMPD3 PLA2G2A PLA2G10 EPHX2 CA1 IDO1 PDE10A PFKFB3 LDHA ALOX15 HSD17B1 NOS1 FDFT1 FDFT1 MAPK1.
hsa00910	Nitrogen metabolism.	12	1.30E-12	CA5B CA13 CA7 CA14 CA6 CA9 CA1 CA1 MAPK1 CDK4 MAPK3 PRKCG PIK3CA.
hsa05223	Non-small cell lung cancer.	17	7.14E-12	STAT3 CDK6 EGFR PIK3CB PRKCB MET ALK JAK3 PRKCA RXRA PIK3R1 AKT1 AKT1 MAPK1 HMOX1 CDK4 MAPK3 PRKCG.
hsa05225	Hepatocellular carcinoma.	20	2.15E-09	PIK3CA CDK6 IGF1R EGFR PIK3CB BCL2L1 PRKCB TERT GSTM1 MET GSK3B MTOR PRKCA PIK3R1 AKT1 AKT1 MAPK1 CYP27B1 MAPK14 MAPK3 CASP3.
hsa04015	Rap1 signaling pathway.	27	1.05E-12	PIK3CA IGF1R EGFR KIT PIK3CB INSR ADORA2B PRKCB MET F2R ADORA2A DRD2 CNR1 SRC RASGRP3 FGFR1 PRKCA PIK3R1 PGF AKT1 VAV1 VEGFA VEGFA CA12 CA2 CA3 CA4 CA5A.

Table 6: Binding affinity, hydrogen bond interaction and non-hydrogen bond interaction of the compound with their protein targets.

Gene ID	Phytoconstituents	BE(Kcal/mol)	Hydrogen bond interaction	Non HBI
MAPK3	Teasterone[a]	-8.6	META125.	CYSA183,VALA56,ILEA48,LEUA173.
AKT1	Quercetin	-8.3	LYSA179,LYSA276,THRA312,ASPA274,	LEUA295.
PRKCA	Quercetin	-7.8	ASPA601,TYRA275,META269.	ILEA610,ALAA271,PHEA261,LYSA604.
IGFR1	Teasterone[a]	-6.7	Nil	TYRA168,TYRA178.
	Clerosterol	-6.7	Nil	LEUA1361,PROA1362,TYRA168,TYRA718.
KDR	Teasterone[a]	-6.3	HISA24	PROA16

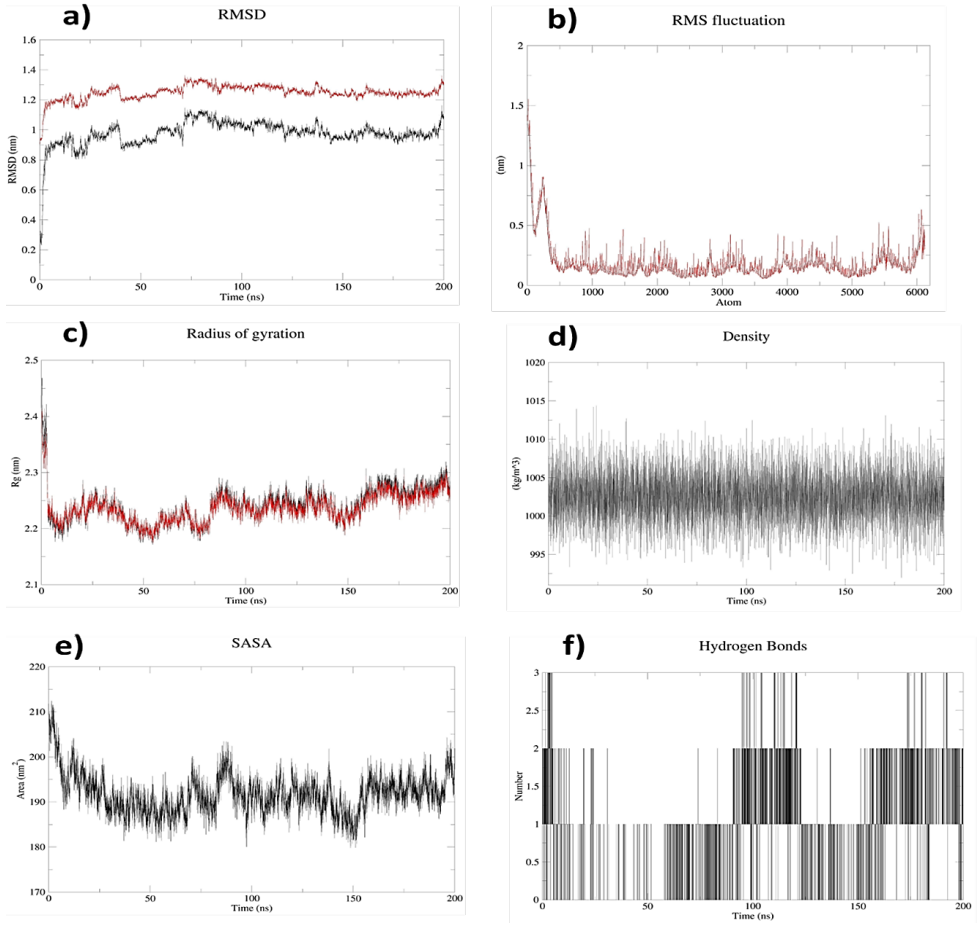

Figure 3: MD Simulation profile of Teasterone[a] with MAPK3 complex where, (a) RMSD of the backbone (Black) and complex (Red), (b) RMSF of c-alpha atoms of complex (black) for Teasterone[a] with MAPK3 (c) Radius of gyration of complex for Teasterone[a] with MAPK3 (d) Density of complex (e) SASA of complex (f) Hydrogen bonds.

(Protein Kinase C alpha): PRKCA is a protein kinase that is involved in signalling pathways. It may influence the signalling networks involved in immune responses and inflammation during tuberculosis infection. KDR (Kinase Insert Domain Receptor, VEGFR2): KDR is a receptor for Vascular Endothelial Growth Factor (VEGF), which is involved in angiogenesis (blood vessel formation). While angiogenesis is not a direct focus of TB research, it is possible that changes in blood vessel formation could have some implications for immune cell trafficking to and from infection sites. MAPK3 (Mitogen-Activated Protein Kinase 3, ERK1): MAPK3 is a member of the MAPK signalling pathway, which is involved in cell proliferation, differentiation and immune responses. It could potentially influence immune cell function and cytokine production during TB infection. The Ras

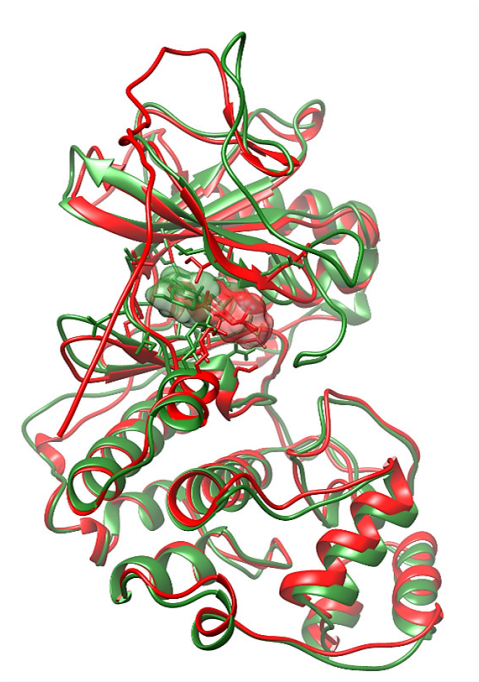

Figure 4: The 3D interaction of Teasterone[a] with MAPK3 (receptor name and compound) at the start (Red) and end (Green) of the simulation.

signalling Pathway is involved in cell growth, proliferation and differentiation, which could play a role in modulating immune cell responses in Tuberculosis (TB). Endocrine resistance is more relevant in cancer treatment, particularly hormone-sensitive cancers, but in TB, it might not have a direct role. The AGE-RAGE signalling Pathway in diabetic complications is implicated, but direct connections between the AGE-RAGE pathway and TB might be less clear. Hypoxia-Inducible Factor 1 (HIF-1) is involved in responses to low oxygen conditions and in TB, where hypoxia can occur within granulomas, the HIF-1 pathway might influence immune cell behaviour and bacterial persistence. EGFR Tyrosine Kinase Inhibitor Resistance is primarily associated with resistance to certain cancer treatments, but its relevance to TB is limited. The PI3K-Akt signalling pathway is crucial for cell survival and growth and in TB; it might affect the balance between pro-inflammatory and anti-inflammatory responses of immune cells. Metabolic pathways play a significant role in providing energy and nutrients for immune responses. TB can alter host cell metabolism and might be influenced by the metabolic state of immune cells. Nitrogen metabolism is not directly associated with TB but could impact immune cell function and responses to infection. Other pathways, such as non-small cell lung cancer and hepatocellular carcinoma, are relevant to liver cancer and not directly related to TB. Rap1 signalling pathway plays roles in cell adhesion, migration and proliferation and in TB; it might impact immune cell migration and interactions within granulomas.

CONCLUSION

In conclusion, the present study delves into the potential therapeutic mechanisms of *Phaseolus vulgaris* phytoconstituents against TB by targeting specific proteins and pathways. Through an exhaustive analysis of 11 documented phytoconstituents, this research identifies key interactions that could offer novel insights into TB treatment strategies.

The study highlights five potential therapeutic targets among a larger pool of protein molecules, namely IGf1r, AKT1, PRKCA, KDR and MAPK3. These targets, previously associated with various cellular processes, emerge as significant candidates in combating TB due to their roles in cell growth, survival, proliferation and immune responses. This connection between immune response regulation and infection control underscores the importance of understanding their impact on TB dynamics.

The predicted interactions extend beyond single proteins, encompassing intricate pathways crucial to cellular function and immune responses. The involvement of these targets in pathways such as HIF-1, AGE-RAGE, PI3K-Akt, Ras and Rap1 signalling pathways hints at their potential influence on immune cell behaviour, metabolic balance and response to hypoxic conditions during TB infection. Furthermore, the study delves into connections with pathways like EGFR tyrosine kinase inhibitor resistance, metabolic pathway, endocrine resistance, non-small cell lung cancer and hepatocellular carcinoma, potentially offering avenues for further exploration in terms of their indirect implications for TB. While some pathways like endocrine resistance and metabolic pathways may have limited direct relevance to TB, their impact on immune cell function cannot be ignored. The complex interplay of these pathways could shape the course of infection, immune responses and treatment outcomes.

Overall, this study provides a comprehensive framework to comprehend the intricate interactions between *Phaseolus vulgaris* phytoconstituents, protein molecules and pathways in the context of TB. The identified targets and associated pathways offer a promising starting point for further research, potentially paving the way for the development of innovative therapeutic interventions aimed at enhancing TB treatment efficacy and improving patient outcomes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TB: Tuberculosis; **MDS:** Molecular Dynamics Simulation; **RMSD:** Root Mean Square Deviation; **RMSF:** Root Mean Square Fluctuation; **RoG:** Radius of Gyration; **SASA:** Solvent Accessible Surface Area; **MW:** Molecular Weight; **MF:** Molecular Formula; **NHBA:** Number of Hydrogen Bond Donors; **NHBA:** Number of Hydrogen Bond Acceptors.

SUMMARY

In this study, we investigated the potential therapeutic mechanisms of phytoconstituents from *Phaseolus vulgaris* against Tuberculosis (TB). TB, caused by Mycobacterium tuberculosis, remains a global health threat, with drug-resistant strains complicating management. We employed a multi-faceted approach, including target identification, compound-gene set enrichment analysis, network pharmacology and molecular docking studies.

The investigation begins with the identification of 151 compounds from *Phaseolus vulgaris*, considering factors like oral bioavailability and drug similarity. Through gene set enrichment and network pharmacology, 11 phytoconstituents are selected based on their drug-like properties. These compounds are assessed for toxicity and three-ascorbic acid, catechin and kaempferol-are found to exhibit non-toxic properties.

Target identification involves querying canonical SMILES using Swiss target prediction and STITCH, along with tuberculosis-related targets from Gene Cards and OMIM Database. The resulting 1056 targets are associated with 197 pathways and 11 pathways directly linked to TB are identified. Among the 1056 targets, IGf1r, AKT1, PRKCA, KDR and MAPK3 are recognized as potential therapeutic targets for TB.

Molecular docking studies focus on compounds with the highest edge count, namely Teasterone[a], Clerosterol, Quercetin and Kaempferol. Docking interactions with TB-related proteins (MAPK3, IGFR1, KDR, AKT1 and PRKCA) reveal potential binding energies and interactions.

Molecular Dynamics Simulation (MDS) assesses the stability of the Teasterone[a] with MAPK3 complex over 200 ns. The analysis includes Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyration (RoG), Solvent Accessible Surface Area (SASA), total density and hydrogen bonds.

The results indicate stability in the Teasterone[a] with MAPK3 complex during MDS, with fluctuations within acceptable ranges. The study emphasizes the potential impact of identified targets and associated pathways on immune responses, metabolic balance and responses to hypoxic conditions during TB infection.

In conclusion, this comprehensive study offers insights into the molecular mechanisms of *Phaseolus vulgaris* phytoconstituents against TB. It identifies potential therapeutic targets and pathways, providing a foundation for further research and the development of innovative interventions to enhance TB treatment efficacy.

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